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Diagnosis of Four Chromosome Abnormalities of Unknown Origin by Chromosome Microdissection and Subsequent Reverse and Forward Painting

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A molecular cytogenetic method consisting of chromosome microdissection and subsequent reverse/forward chromosome painting is a powerful tool to identify chromosome abnormalities of unknown origin. We present 4 cases of chromosome structural abnormalities whose origins were ascertained by this method. In one MCA/MR patient with an add(5q)chromosome, fluorescence in situ hybridization (FISH), using probes generated from a microdissected additional segment of the add(5q) chromosome and then from a distal region of normal chromosome 5, confirmed that the patient had a tandem duplication for a 5q35-qter segment. Similarly, we ascertained that an additional segment of an add(3p) chromosome in another MCA/MR patient had been derived from a 7q32-qter segment. In a woman with a history of successive spontaneous abortions and with a minute marker chromosome, painting using microdissected probes from the whole marker chromosome revealed that it was i(15)(p10) or psu dic(15;15)(q11;q11). Likewise, a marker observed in a fetus was a ring chromosome derived from the paracentromeric region of chromosome 19. We emphasize the value of the microdissection-based chromosome painting method in the identification of unknown chromosomes, especially for marker chromosomes. The method may contribute to a collection of data among patients with similar or identical chromosome abnormalities, which may lead to a better clinical syndrome delineation. © 1996 Wiley-Liss, Inc.

KEY WORDS: chromosome microdissection, chromosome painting, chromosome aberration, marker chromosome

INTRODUCTION

Chromosome rearrangements are sometimes difficult to be unequivocally characterized. De novo chromosome structural abnormalities or supernumerary marker chromosomes of unknown origin cannot always be accurately diagnosed by standard cytogenetic methods. Recent development of fluorescence in situ hybridization (FISH)-based reverse and forward chromosome painting using DNA probes from a microdissected chromosomal region has made it possible to approach the diagnosis of such abnormalities [Carter et al., 1992; Deng et al., 1992; Ohta et al., 1993; Viersbach et al., 1994]. FISH on normal chromosomes using a probe pool generated from an aberrant chromosome itself is reverse painting, and an opposite approach using a probe pool from a normal chromosome of interest is the forward painting. The accuracy of this targeted painting has been proven in studies not only on constitutional chromosome structural abnormalities [Carter et al., 1992; Deng et al., 1992; Ohta et al., 1993; Viersbach et al., 1994] but also on the abnormalities in cancers [Meltzer et al., 1992; Guan et al., 1994]. Since reverse/forward chromosome painting allows rapid analysis of the abnormalities, the resulting data are useful for genetic counseling. This paper describes the diagnosis of 4 cases of chromosome abnormalities with the chromosome band-specific painting techniques.

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MATERIALS AND METHODS

Chromosome Preparation for Microdissection and for FISH

Procedures for chromosome preparations were modifications of those described previously [Ohta et al., 1993]. Four subjects were studied. Chromosome abnormalities of all but case 3 were de novo type. Case 1 was a patient with MCA/MR and had a 46,XX,add(5)(q35) karyotype (Fig. 1a). Case 2, a patient with MCA/MR, had an add(3)(p26) of which an additional segment was unknown (Fig. 1b). Case 3 was a 29-year-old phenotypically normal pregnant woman who had had 2 successive spontaneous abortions. The woman had an additional minute marker chromosome of unknown origin (Fig. 1c), which was inherited from her phenotypically normal father. The marker consisted of a centromere and two arms, looking like inverted duplication of an acrocentric chromosome. Case 4, a 10-week-old fetus, was referred for prenatal diagnosis. A small ring-like marker chromosome was found in cultured chorionic villus cells (Fig. 1d). The pregnancy was terminated at 18 weeks of gestation. Cultured Epstein-Barr virus-transformed lymphoblastoid cells from cases 1 and 2, peripheral blood lymphocytes from case 3, and chorionic cells from case 4 were treated with 0.05 μg/ml Colcemid 2 hr prior to harvest, and then with hypotonic solution containing 0.2% NaCl and 0.2% KCl. A cell suspension from each sample was stored at −20°C until use. A fixed cell suspension (30μl) was mixed with acetic acid (10 μl), and then immediately spread onto a clean glass slide. Non-aged chromosomes were GTG-banded and used immediately for microdissection. R-banded metaphase chromosomes from karyotypically normal individuals were also used for reverse painting.

Microdissection and PCR Amplification of Chromosomal DNA

Procedures were those described previously [Hirota et al., 1992; Jinno et al., 1992; Deng et al., 1992; Ohta et al., 1993]. In short, 20 to 60 chromosome pieces were removed from a defined chromosome region with a fine glass needle under an inverted microscope. After DNA extraction with proteinase K/phenol/chloroform treatments, the DNA was digested with *Sau*3AI. Further steps, including DNA ligation to a linker/primer set, and polymerase chain reaction (PCR) amplification with the linker-primer method, were carried out as described previously [Hirota et al., 1992; Jinno et al., 1992]. The sequences of a 10mer linker DNA (MboL1) was 5′-GATCCATGTC-3′, and those of a 24mer primer DNA (NlaM1) 5′-CGGGAATTCTGGCTCTGCGACATG-3′ [Jinno et al., 1992]. PCR was performed for 31 cycles with denaturation at 93°C for 2 min, annealing at 62°C for 2 min, and extension at 72°C for 2 min. The mean size of the PCR products ranged from 200 to 500 bp.

Chromosome Painting

A small volume (4 μl) of the PCR product underwent a second round of PCR to be biotin-labeled. After 11 cycles of the second-labeling PCR in a reaction mixture containing 125 μM biotin-16-dUTP and 100 μM dTTP, PCR products were combined for use as a probe pool, together with unlabeled human Cot-1 DNA (GIBCO BRL, U.S.A.) used as a competitor. Hybridization was performed at 37°C overnight, followed by washing in 50% formamide, 2 × SSC at 37°C, then in 2 × SSC and 1 × SSC at room temperature each for 5 min, and finally in 4 × SSC at room temperature for 5 min. FISH signals were detected with FITC-conjugated avidin, and chromosomes were counterstained with propidium iodide. Photomicroscopy was performed under a fluorescence microscope equipped with filter combinations B-2A or B-2E (Nikon, Japan).

RESULTS

Case 1

With a probe pool made from 30 microdissected chromosome pieces which covered the excess segment of the add(5q) chromosome, FISH on metaphase chromosomes from a karyotypically normal person showed FITC signals on the distal long arms of both homologous chromosomes 5. FISH using another probe pool generated by microdissection of a distal 5q region of the normal person demonstrated a double-band signal and a one-band signal on the aberrant and normal chromosome 5 of the patient, respectively (Fig. 2a). The signal on the R-banded normal chromosome 5 corresponded to a q35-qter segment, and the visually apparent increased signal on the abnormal chromosome 5 was consistent with a partial duplication of the segment. Thus, the patient's further karyotype was 46,XX,dup(5)(q35qter).

Case 2

When using PCR products as a probe pool generated from 28 dissected chromosome fragments containing the additional segment of the add(3p) chromosome, 2 FISH signals appeared at the distal 3p and 2 signals at a distal 7q (7q32-qter) on R-banded metaphase chromosomes from a normal person (Fig. 2b). Then, a distal long arm region of normal chromosomes 7 was dissected and this probe pool was hybridized to the patient's chromosomes. As a result, FISH signals appeared at a distal region of the add(3p) as well as at the distal 7q (Fig. 2c). Combining the FISH results with GTG-banded patterns of the add(3p), we concluded that the karyotype of the patient was 46,XY,der(3)t(3;7)(p26;q32), indicating partial trisomy for 7q32-qter.

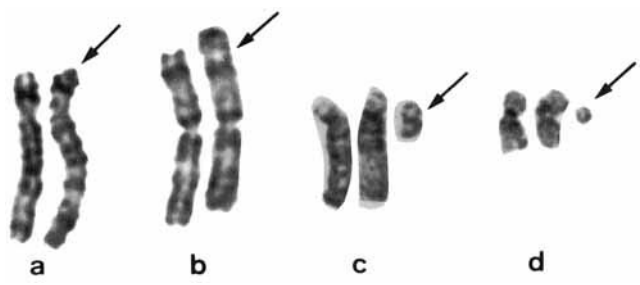

Fig. 1. Partial GTG-banded karyotypes of cases 1–4 (**a–d**). Arrows show abnormal chromosomes.

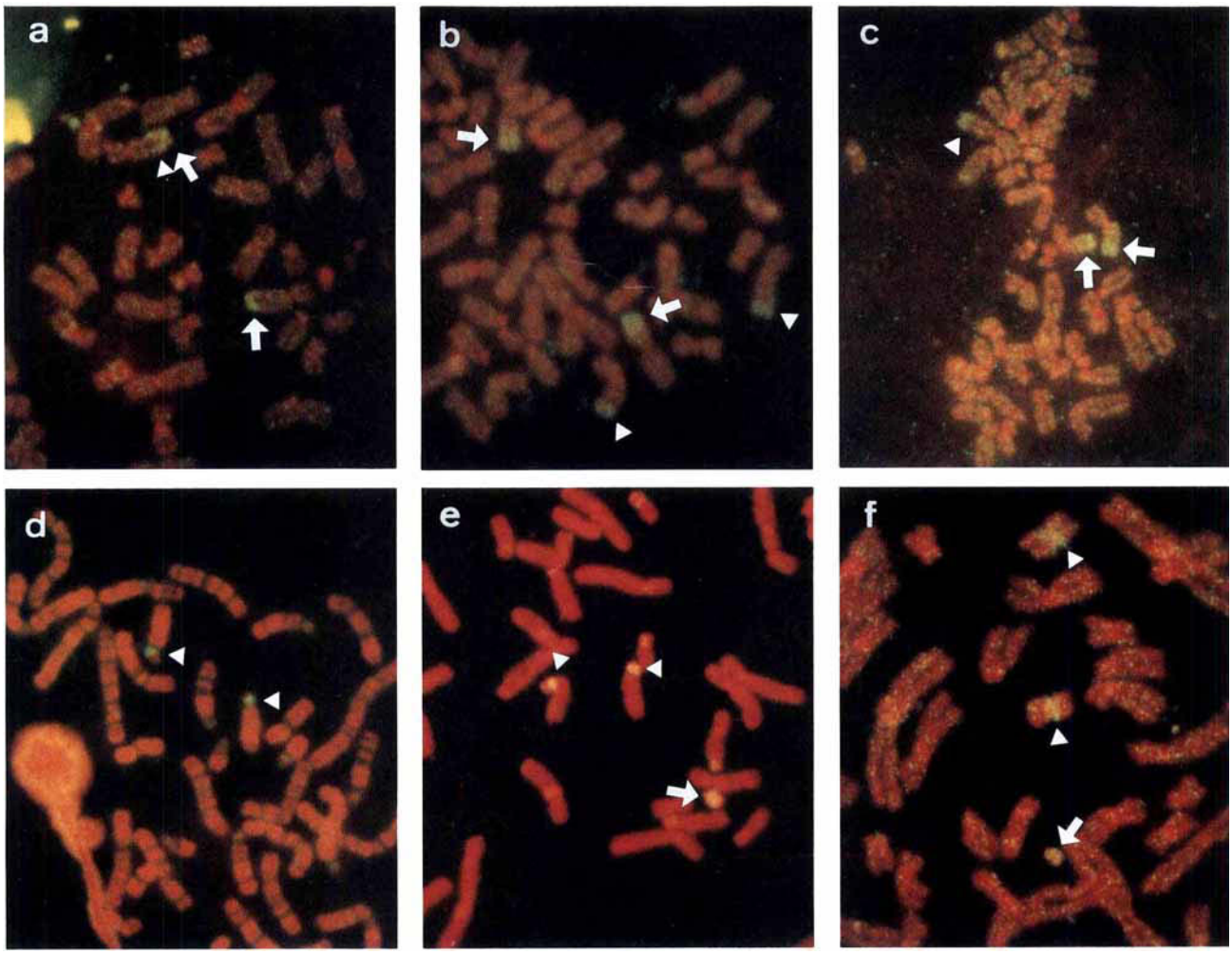

Fig. 2. Chromosome painting using probe pools generated from DNA obtained by microdissection of defined chromosomal regions. FISH on case 1 chromosomes using normal 5q-derived probes **(a)**; those on normal chromosomes using the add(3p) probes of case 2 **(b)** and on case 2 chromosomes using normal 7q probes **(c)**; those on normal **(d)** and case 3 chromosomes **(e)** using probes from the marker of case 3; and that on case 4 chromosomes using probes from the marker of the patient **(f)**. Arrows and arrowheads show FITC signals on chromosomal regions corresponding to dissected localizations and those on other regions, respectively.

Case 3

FISH with a probe pool made from 60 pieces of the entire marker chromosome of this patient strongly painted a short arm region of normal chromosomes 15 (Fig. 2d). Another FISH demonstrated bright fluorescence signals on the marker chromosome as well as 15p of the patient (Fig. 2e). Thus, the marker chromosome was finally diagnosed as either i(15)(p10) or psu dic(15;15)(q11;q11).

Case 4

A probe pool was made from 30 pieces of the entire marker chromosome. FISH with the probe on the patient's chromosomes gave signals on the paracentromeric region of chromosomes 19 as well as on the marker chromosome (Fig. 2f). Reverse FISH on chromosomes from a normal person also presented signals at the region of chromosome 19. Thus, the karyotype of the fetus was interpreted as 47,XY,+r(19)(p12q12).

DISCUSSION

We presented in this paper an efficient approach of the reverse/forward chromosome painting to the identification of de novo translocations and marker chromosomes. We adopted a 2-way painting, first, reverse painting using a probe pool generated directly from aberrant chromosomes or chromosomal regions and second, forward painting using a probe pool from the regions in normal chromosomes, supposed to be corresponding to the aberrant regions. With this method, the origin of abnormal chromosomes were accurately ascertained in all the 4 cases studied. Case 1 was finally diagnosed to have tandem duplication of a 5q35-qter segment, because a double-size FISH signal appeared

on the abnormal chromosome, indicating that the painting can detect not only the position of probes to be tested but also the content of genetic materials in a chromosome. In case 3, the origin of an inherited marker chromosome was confirmed to be the short arm region of chromosome 15. Although the paracentromeric region of chromosome 15 contains highly repetitive sequences common to those of other acrocentrics, it was possible to obtain chromosome 15-specific FISH signals when the amount of Cot-1 DNA used as a competitor was increased to 2 μg/ml. Thus, under a proper hybridization condition, FISH can reveal a cognate chromosome of a marker chromosome by showing distinct clusters of fluorescent signal or spots, even though the paracentromeric region of a particular acrocentric is involved. Case 3 had a history of successive spontaneous abortions, and the additional marker chromosome may have caused the abortions. Some degree of association has been observed between such marker chromosome carriers and infertility [Buckton et al., 1985]. Alternatively, the marker chromosome in this woman was just observed coincidentally.

Marker chromosomes are referred to as small chromosomes of unknown origin and they are often associated with developmental abnormalities [Callen et al., 1992; Plattner et al., 1993; Blennow et al., 1992; Cheng et al., 1994; Viersbach et al., 1994]. However, since the ascertainment of the origin of most markers is difficult, they have not been assigned to a particular syndrome. Thus, data given by the reverse painting will contribute to the collection of patients with similar or identical marker chromosomes [Viersbach et al., 1994]. Consequently, a comparison of clinical manifestations among such patients may allow the recognition of new syndromes. Furthermore, the regions or genes proven to be involved in such markers provide an interesting approach to the understanding of developmental abnormalities. During prenatal screening, supernumerary marker chromosomes are often observed and this is one of the significant problems for subsequent genetic counseling. The overall incidence of these kinds of markers in amniocentesis materials is estimated to be much higher than that in newborn series [Hsu, 1986; Warburton, 1991]. It seems that, at least in the amniocentesis population, there are more de novo marker chromosomes than inherited ones [Hsu, 1986]. Reverse chromosome painting as adopted in the diagnosis for our case 4 is a powerful method for the identification of such markers observed in prenatal diagnosis materials.

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